Studies on the structure and biosynthesis of posttranslationally modified isoforms of the bacteriocin BacSp222

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BacSp222 is a linear, 50-amino acid long peptide produced by a zoonotic strain *Staphylococcus pseudintermedius* 222. BacSp222 serves both as a bacteriocin as well as a virulence factor: it kills Gram-positive bacteria and is cytotoxic as well as immunomodulatory to selected eukaryotic cells. (Previously published in: Wladyka B. et al. (2015) Sci. Rep. 5, 14569, Nowakowski M. et al. (2018) Int. J. Biol. Macromolecules 107, 2715–2724.) We have recently discovered that BacSp222 is produced in three different posttranslationally modified isoforms. In the present research, we focused on the mechanism of modification and on the influence of modifications on the structure and activity of BacSp222. The identity of posttranslational modifications was determined by Edman degradation and mass spectrometry, and these studies showed that BacSp222 is produced as an unmodified isoform i1, an isoform i2 containing one butanedioic (succinyl) group, and an isoform i3 with two butanedioic groups. These modifications occupy epsilon-amino groups of lysine residues. We demonstrated that succinylation is a nonenzymatic reaction - in contrast to desuccinylation, which is a NAD- and cytoplasmic enzymes-dependent process. The only physiological donor of the butanedioic group able to modify BacSp222 is succinyl-coenzyme A. The nuclear magnetic resonance and circular dichroism studies did not show any significant differences between the conformation of i1 and i2. On the other hand, the antibacterial activity of i2 and i3 is significantly lower than i1. The level of modified isoforms depends on environmental factors such as different sources of carbon, culture temperature, and pH of the medium. In sum, BacSp222 is the first described bacteriocin which has posttranslationally succinylated lysine residues. Our results suggest that these modifications protect producer cells against the autotoxicity of the excreted peptide. Funding: National Science Centre, Poland (grant No 2018/31/B/NZ3/01226).